BIOCHEMISTRY

CONSTRUCTION OF A FULL-LENGTH BOVINE DEOXYHYPUSINE SYNTHASE INTO AN EXPRESSION VECTOR, pET11a.

Jonathan T. Ferguson, Jenq-Kuen Huang*, Shu H. Tsai, Vasudevroy Bhuvanagiri, Srisivaprasad Doddapaneni, S.S.R. Prasad Lakkaraju Venkata, Baburam Sedai, Nanjia Yu, Anupama Boddapati, and Lisa Wen*. Department of Chemistry, Western Illinois University, Macomb, IL 61455. J-Huang3@wiu.edu, L-Wen@wiu.edu.

Mature eukaryotic initiation factor-5A (eIF-5A) is the only protein known to contain the unusual amino acid hypusine, (N-(4-amino-2(R)-hydroxybutyl)lysine). Hypusine synthesis in mature eIF-5A is a unique two-step posttranslational modification catalyzed by deoxyhypusine synthase (DS) and deoxyhypusine hydroxylase (DH). The synthesis of hypusine, and therefore of mature eIF-5A, is important for the biological activity of eIF-5A. High levels of eIF5A have been found to exist in tumor cells, and it also acts as a cofactor of the Rev transactivator protein in HIV-1-infected cells. Inhibitors of either of DS or DH have been shown to exert antiproliferative and antiretroviral effects. Therefore, these two enzymes are regarded as potential targets for antiproliferative therapy. Detailed structural-functional studies of DS or DH will allow development of more specific inhibitors of these enzymes for use to control hyperproliferative diseases.

Both DS and eIF-5A precursor proteins are essential in developing assays for DH. This project is aimed at construction of bovine deoxyhypusine synthase cDNA into an expression vector and production of recombinant bovine DS for use in bovine deoxyhypusine hydroxylase activity assays. Full-length cDNA of bovine DS has been amplified by polymerase chain reaction (PCR). The amplified DNA fragment has been inserted into expression vector, pET11a, at Bam HI site. The orientation of the insert is being examined by PCR using appropriate primers. Recombinant fusion protein will be expressed and will contain a T7-tag protein on its N-terminus which can be purified by a T7-tag antibody affinity column. Expression of soluble recombinant bovine DS will facilitate our purification and monitoring of deoxyhypusine hydroxylase activities.

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